

CURRENT UPDATE ON CANINE PARVOVIRUS IN VETERINARY FIELD ASPECT

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ABSTRACT

Huge numbers of puppies are killed by parvovirus infection and this disease is transmitted by inanimate object as well as insect and rodents. Parvovirus disease can be controlled by modified live virus vaccines, which are safe and effective to control by adopting the vaccination protocol. CPV-2c strain not easily detected in laboratory test and now no vaccine are available to protect against that virus strain. So symptomatic treatment is done by the veterinarian and best is vaccination, which play important role in control the disease mainly in puppies.

KEY WORD: Parvovirus

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INTRODUCTION

Canine parvovirus infection is mainly the diseases of young pups and adult dogs. Binn *et al.*, (1970) first recovered a small antigenically related virus, from faecal specimens of several normal dogs and characterized it as 'Minute virus of canine' (MVC) or canine Parvovirus (CPV) type-1. Appel *et al.* (1978), reported the first epizootic of a new enteric disease of dogs in which, vomiting and diarrhea were the prominent symptoms. Similar syndrome in young puppies caused by myocarditis and congestive heart failure, was simultaneously reported (Hayes *et al.*, 1979). A parvovirus was demonstrated and isolated from these cases and classified as canine parvovirus type 2 (CPV-2). At present, CPV-2 is the most common agent causing clinical disease, throughout the world including India. (Murphy *et al.*, 1999).

Canine parvovirus 2 (CPV2), a non- enveloped icosahedral virus, with an approximate diameter of 20 nm, is the member of genus Parvovirus of the family, *Parvoviridae* virus is a single-stranded DNA virus, which have 5,200 nucleotides. The virus contain two structural (VP1 and VP2) and two non-structural (NS1 and NS2) proteins. (Notomi *et al.*, 2000).

The CPV replicates in enteritis in dogs of all ages groups and causes myocarditis in young pups. The virus infects rapidly and divide in host cells viz. bone marrow cells, intestinal cells, lymph system cells, and fetal cells. In vitro, MDCK, CFRK (Gupta *et al.*, 2005) and A- 72 (Yilmaz *et al.*, 2005) cells were used, for its isolation and characterization.

CPV withstands in the environment and dog keeping house, and transmitted indirectly or directly in dogs and cats. The major impact of CPV on humans is economical and psychological. Serological survey conducted among kennel and laboratory workers, have failed to find any evidence that CPV is transmissible to human. Hence CPV is not considered as pathogen with zoonotic potential.

Canine parvovirus (CPV) is responsible for acute gastroenteritis in pups, with a high rate of mortality

(Carmichael and Binn, 1981). Parvovirus infection in dogs caused by CPV-2, has become an alarming problem in India, causing high morbidity and low mortality. Canine Parvovirus disease was recognized by vomiting and diarrhea in pups with morbidity rate upto 100% and frequent mortality rate up to 7.5 to 24 percent (Sahu *et al.*, 2007), that may reach upto 69 percent in young pups (Horner, 1983).

There are two canine parvovirus strains viz. CPV2a and CPV2b, which were identified in year 1979 and 1984, respectively (Shackelton *et al.*, 2005). Most of the dogs are infected by above two strains (Martella *et al.*, 2005). Decaro *et al.*, 2006 discovered a third type, CPV2c (a Glu-426 mutant) in Italy, Vietnam, and Spain.

GENOMIC CHARACTERIZATION

In 1985, the nucleotide sequence of the coat protein gene of canine parvovirus was studied by Rhode (1985). Later, Reed *et al.*, (1988) studied the nucleotide sequence of the entire genome of canine parvovirus and its organization. The CPV genome is of 5323 nucleotide in length, having two major open reading frames (ORF). The ORF in the 3' half of the genome that encodes the non structural proteins NS1 and NS2, while the ORF in 5' half of the genome encodes the structural protein VP1 and VP2. The structural and non structural proteins are initiated from separate promoters. The mRNA of the non structural and structural proteins has co-terminal poly (A), addition sites and spliced to allow alternate templates for protein synthesis.

VP1/VP2 Gene

Location of VP1 gene, is in 2253 bases whereas, VP2 gene is found 1755 base. The VP2 monomer comprised of an 8-stranded anti parallel β -barrel, with large loops inserted between the β -strands making up most of the exposed surface of the capsid. Surface feature of the capsid included a raised area (spike), surrounding the three-fold axis of symmetry, a depression (dimple) spanning the two-fold axis of symmetry, and a further depression area (canyon) surrounding the five fold axis of symmetry of capsid. Two primary antigenic sites on the three-fold spike of the virus, were defined using monoclonal antibodies (Mab) (Strassheim *et al.*, 1994), although epitopes have also been defined by peptide mapping, in other regions of the capsid (Langeveld *et al.*, 1993). There are three locations on capsid, which can affect canine host range on three fold spikes, which are separated by 25 to 30 Å each other, that is ultimately affects on the flexibility of loops, interactions in virus-receptors or uncoating of capsid (Llamas-Saiz *et al.*, 1996).

Hemagglutination Property

Heamagglutination is an important property of canine parvovirus. CPV bind to pig erythrocytes, rhesus monkey and African green monkey (Gagnon and Povey, 1979). Senda *et al.*, (1986) reported that, observed RBC of horse/ dog/ sheep/mouse etc are agglutinated by CPV. Hemagglutination (HA) property of CPV and FPV were different, due to binding ability to the specific sialic acids. The first difference is that, the FPV isolates haemagglutinate, only in the buffers at pHs below 6.6, while the CPV isolates haemagglutinate at higher pHs up to at least pH 7.5 (Parrish *et al.*, 1998). The HA specificity of the virus, appears to be specific for the sialic acid N-glycolyneuraminic acid, which is present only on feline erythrocytes of the A blood group (about 70% of almost all the breeds of domestic cat), but now present on the erythrocytes of most breeds of dogs.

HOST RANGE

These infect warm-blooded animals ranging from domestic fowl to human. These are among the most antigenic

infectious agents and active infection of animal results in relatively high and persistent antibody levels and long lasting passive immunity in offspring's. Parvo virus responsible for infection is usually sub-clinical, but may affect the fetus, goose parvovirus causes lethal diseases in goslings. Rodent parvoviruses are used as a model in studies of the pathogenesis of certain fetal abnormalities, and are common contaminants of cultured rodents cells and tumors. In humans B19 parvovirus has been incriminated as the cause of a common exanthematous disease, erythema infectiosum. Parvoviruses have been isolated from chickens, rabbits and an equine fetus, but their role in causing disease in these species has not been established.

SYMPTOMS

- Depression
- Animal suffering from fever
- Animal may be dehydrated and anorectic
- Severe bloody diarrheas
- Vomiting and restlessness

TRANSMISSION

The virus is transmitted to susceptible host by the faecal-oral route. In recent time it is believed that, one virus does not infect the other species. CPV 2 can undergo mutation to infect the other. FPV is similar to CPV and this similarity is up to 80 per cent or more than that (Ikeda *et al.*, 2000). As compared to dog, this virus (CPV-2) is found more in cats. The virus is not reported in birds and human being, but both act as a carrier, which ultimately infect the dogs and cats (Murphy *et al.*, 1999).

- Mochizuki *et al.*, in 1996, isolate the CPV from faecal sample of cat, manifesting clinical signs of feline panleukopenia and suggested FPLV and CPV, which is transmitted between cats and dogs and also canine disease in other adventitious host.

DIAGNOSIS

Diagnosis of CPV infection is based on detection of virus by electron microscopy, Serological tests and by molecular techniques. Tentative diagnosis can be made on the basis of the clinical symptoms and history but for confirmatory diagnosis the clinical samples of choice are faeces and pieces of tonsil, spleen and lymph nodes.

The virus in the faeces can be detected by HA test, with pig erythrocytes. (Carmichael *et al.*, 1980; Durigon *et al.*, 1987; Senda *et al.*, 1986) and ELISA test. Isolation of the virus from faeces and other tissues can be made, during the acute stage of the disease. The virus particles in the faeces can also be demonstrated under electron microscopy, by negative staining. ELISA test is useful to detect the viral antigen in faeces and clinical samples (Durigon *et al.* 1987; Teramoto *et al.*, 1984).

Isolation of virus is the most sensitive method, for detecting low concentrations of virus in fecal sample. The virus can be isolated in primary canine cells, or in several different canine or feline cell lines like canine A72 cells and crandell

feline kidney cell line (CRFK). The MDCK canine kidney cell line is reported to be, not susceptible to CPV2 infection.

The virus specific antibodies in serum samples can be detected by HI and ELISA tests. The HI test is reliable, when high antibody titre (1:100) is present. Presence of nonspecific inhibitors of HA test may be present in some sera, which may give false positive reaction and this can be removed by treating the sera with kaolin.

Virus Isolation

The isolation and identification of virus has been a consistent and most appropriate method. The CPV was successfully isolated and identified, using cell cultures and cell lines such as in MDCK and CFRK (Gupta *et al.*, 2005) and A-72 (Yilmaz *et al.*, 2005) cells were used for its isolation and characterization. CPV infect not only the canine cell line, but also infect the feline cell line whereas, FPV only infect the feline cells.

Electron Microscopy

Negative stain electron microscopy has been used to detect CPV particles (Hamilton *et al.*, 1995). Cho *et al.*, (2004), found electron microscopy to be 90 percent sensitive and 40 percent specific in detecting CPV in faecal samples. Although EM is highly specific and sensitive, it often is too time consuming and expensive for routine use in a clinical setting Cho *et al.*, (2006).

Serological Tests

The simplest and reliable procedure for laboratory diagnosis of canine parvovirus infection, is the detection of CPV antigen in faecal extracts by hemagglutination test using pig or rhesus monkey red blood cells at pH 6.5 and at 4°C temperature (Kumar *et al.*, 2003). The specificity this hemagglutination is determined by titrating the fecal specimen in parallel in the response of normal and immune dog serum. Fecal sample from dogs with acute enteritis may contain up to 20,000 hemagglutinating units of virus, equivalent to about 10⁹ virions, per gram (Murphy *et al.*, 1999).

Kumar *et al.*, (2003) studied the haemagglutinating activity of canine parvovirus to standardize the haemagglutination (HA) test for the diagnosis of canine parvovirus (CPV) infection in dogs using erythrocytes of different species. The HA titre of <1:8 was considered negative. The authors suggest that the HA test can be used easily and successfully for the detection of parvovirus in faecal samples of dogs excreting the virus.

Timbol *et al.*, in 1983 made the diagnosis of canine parvovirus enteritis by the combined use of hemagglutination and hemagglutination inhibition tests on fecal specimens of suspected cases of canine parvovirus enteritis.

Other serological tests used include ELISA (Phukan *et al.*, 2005), Dot Immunobinding (DIA) (Sanousi, 1990), Counter current immunoelectrophoresis (CIE) (Joshi *et al.*, 2000), Immune Electron Microscopy (IEM) (Mochizuki *et al.*, 1984), Agar gel immunodiffusion test (AGID) (Gunaseelan *et al.*, 1993), Fluorescent Antibody Technique (FAT) (Joshi *et al.*, 1996), Single Radial Haemolysis test (SRHT) (Fastier, 1981), Latex agglutination test (Subhashini *et al.*, 1997) and Co agglutination test (Singh, 2002). Singh (2002) has compared HA, AGID, CIE, Single radial immunodiffusion (SRID), DIA, ELISA and Slide Coagglutination tests for detection of CPV antigen in faecal samples and reported that the ELISA was most sensitive while HA and Coagglutination has almost similar sensitivity. The authors advocated coagglutination test to be specific and easy to perform while HA test to be good for screening the field samples but not a confirmatory test. AGID a CIEP tests were found to be specific but less sensitive.

Molecular Techniques

A battery of molecular techniques has been in use for detecting CPV in samples. Insitu hybridization (ISH) was standardized by Yoshio *et al.*, (1984) and Cho *et al.*, (2004) for detection of CPV in faecal samples. Cho *et al.*, (2004) while comparing ISH with PCR and EM, found that the PCR has highest sensitivity, whereas ISH and PCR both were rapid in virus detection. Cho *et al.* (2006) used ISH to detect CPV in Paraffin embedded tissue samples. Meerarani *et al.*, (1998), used Slot Blot Hybridization using non radioactive probes and found it sensitive and specific. The method was reported to be more sensitive than HA.

Polymerase chain reaction has acquired wide acceptance for laboratory diagnosis of virus infections because of its sensitivity and specificity (Gupta *et al.*, 2006). The method is more useful since an appreciable quantity of virus is shed in faecal samples. Silverstein *et al.*, (2003) recommended that, the PCR can be used later in the disease, when potentially fewer viruses are being shed in the feces that may not be detectable by EIA. Shunck *et al.* (1995) developed a simple touchdown PCR, for CPV and FPV detection.

Loop Mediated Isothermal amplification technique was standardized by Cho *et al.*, 2006, for detecting the CPV DNA in faecal samples. The LAMP technique offers several advantages over PCR analysis, for the detection of CPV-DNA such as, the assay requires only 2 hours to complete and is a sensitive method that can amplify a few copies of DNA to about 10⁹ molecules, in 1 hour under isothermal conditions. When used to detect CPV DNA in a control sample, the LAMP technique had 100-fold higher analytical sensitivity than PCR analysis (10⁻¹ TCID₅₀/ml versus 10 TCID₅₀/ml).

PREVENTION AND DECONTAMINATION

A puppy that recovers from CPV2 is immune to re-infection, for at least 20 months. There are good co-relations between serum antibody titre, determined by HI or virus neutralization test and the resistance to infection. Attenuated live vaccines are available, which may be given at 6.9 and 12 week age and revaccination carried out annually. However many puppies continue to break with the disease, between their first and second or second and third immunization, or even after the entire vaccine series is complete. Primary cause of failure, of most CPV 2 vaccine is, interfering level of maternally derived antibody (MDA). The presence of MDA, the ability of a vaccine to elicit a serological response has been found to be related to antigenic mass in the dose of the vaccine. Experimental vaccine containing optimum level of CPV-2b induced seroconversion rate of 95 per cent in puppies, with serum neutralization titre of 1: 128. (Information Sheet Canine Parvovirus, 2008-2009).

CPV infection is quite common and the disease is usually controlled, using a live or inactivated vaccine, although maternally derived antibodies may inhibit the replication of live attenuated vaccines in puppies having temporary immunity. Commonly, CPV causes disease in unvaccinated 1-6 month-old pups (Elia *et al.*, 2005). Twelve month old age dogs are not susceptible to CPV infection, because of pre-vaccination or by the infections with a field virus strain (Truyen 2006). Untreated cases of CPV2 will have a mortality approaching 91%. With aggressive therapy, survival rates may approach 80-95% (Prittie *et al.*, 2004).

TREATMENT

There is no specific treatment against this viral infection. Antibiotics like tetracycline; chloramphenicol may be administered for 5-10 days. Attempt should be made to prevent secondary bacterial invasions.

Symptomatic treatment should be done to control diarrhoea. Preparation containing kanamycine sulphate, pectin; bismuth sub carbonate and activated attpulgite may be tried.

Dehydration should be controlled with ringer lactate or dextrose, with sodium chloride (NaCl). In severe blood looses, plasma volume expander may be used.

Canine parvovirus strain (CORNELL 780916) modified by passages on cell culture is commonly used as a vaccine against the disease. The vaccine is given by I/M or S/C route followed by second injection after 12th weeks (Macintire *et al.*, 2004).

CONCLUSIONS

Canine parovirus is largely prevailing in dog and it appears to be a predominant cause of gastroenteritis in dogs. This canine parvovirus also affects the wild range of wild animals. A clinical sign of parvoviral disease was seen in captive and free ranging coyotes. The CPV2 was also circulates among domestic as well as canids. Vaccinated pups are usually protected from this disease and from infection. Sometimes vaccination against canine parvovirus does not provide foolproof coverage against the disease and infection in pet dogs, one reason of failure of vaccination may be the mismatching of the vaccine strain and outbreak strains of CPV. This difference may be due to the mutation in virus and new alleles that changes the strain and properties of the virus. So, properly vaccinate the animal along with adopting serosurveillance programme to totally control the disease in dog and cat population.

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